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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. |
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| 09/320,609 | 05/26/99 | WILUSZ J | 601-1-088N |

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| SIU,S | |
| **ART UNIT** | **PAPER NUMBER** |
| 1631 | 13 |

**DATE MAILED:** 12/26/00

**Please find below and/or attached an Office communication concerning this application or proceeding.**

**Commissioner of Patents and Trademarks**

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/320,609 | WILUSZ ET AL. | |
| | Examiner | Art Unit | |
| | Stephen Siu | 1631 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *30 October 2000* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,2,4-6 and 9-55* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *18-20 and 48-50* is/are allowed.

6)☒ Claim(s) *1,2,4-6,9-17,21-47 and 51-55* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

**Attachment(s)**

15) ☒ Notice of References Cited (PTO-892)
16) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *10* .
18) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
19) ☐ Notice of Informal Patent Application (PTO-152)
20) ☐ Other: .

## DETAILED ACTION

This is in response to Applicant's amendment received October 30, 2000 (paper number 11).

The rejection of claims 21-45 under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention as cited in the Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's arguments.

The rejection of claim 38 under 35 U.S.C. 112, second paragraph, as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claims 1-2, 8-10, 12-15, 21, 24-30, and 51-52 under 35 U.S.C. 102(b) as being anticipated by Bernstein as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claims 1, 4-6, 12, 14, 16-17, 21-25, 28 and 55 under 35 U.S.C. 102(b) as being anticipated by Krikorian as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claim 46 under 35 U.S.C. 103(a) as being unpatentable over Bernstein in view of Brewer and in further view of Krikorian as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claims 31-32 and 44-45 under 35 U.S.C. 103(a) as being unpatentable over Bernstein in view Chen in further view of any one of Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, or Liu as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claim 47 under 35 U.S.C. 103(a) as being unpatentable over Bernstein in view of Brewer and in further view of Krikorian as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

The rejection of claims 53 and 54 under 35 U.S.C. 103(a) as being unpatentable over Bernstein in view of Krikorian as cited in the Office Action mailed April 26, 2000 (paper number 9) is withdrawn in view of Applicant's amendment and arguments.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 2, 4-6, 9-17, 21-45, 46-47 and 51-55 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bernstein (Molecular and Cellular Biology, Feb. 1989, Vol.9, No.2, pages 659-670) in view of Beaumont (US Pat 5264372, 11/23/93, filed 3/15/91), Krikorian, Brewer and any one of Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, or Liu.

Bernstein teaches an in-vitro mRNA decay system and its use in a method in which mRNA molecules with poly(A) tracts are shortened with intermediates showing deadenylation of mRNA (page 661, col.2, paragraph 2). The system contains cytoplasmic extract that is depleted of activity of proteins that bind polyadenylate (see abstract). Bernstein obtains the extract by isolating ribosomes, extracting components and centrifugating to obtain a supernatant (RSW).

Bernstein further teaches alternate methods of depleting extract of proteins that bind polyadenlyate and demonstrates the methods of adding competitor poly(A) mRNA, by passing the extract through a poly(A)-Sepharose column (page 661, col.1, last line), or by adding antibody to the protein (page 663, col.2, top). Bernstein demonstrates the addition of agents to the system to observe stabilizing effects by adding cytoplasmic protein and observing the change in stability of the mRNA (page 662, col.1).

Bernstein does not teach centrifugation of the extract at 100,000 x g for one hour.

Beaumont (US Pat 5264372, 11/23/93, filed 3/15/91) teaches centrifugation at high speeds (100,000 x g for one hour) and demonstrates the effectiveness of such centrifugation at separating components in a biological sample (col.14, lines 37-50).

Bernstein does not teach the use of nucleotide triphosphate in the system of mRNA turnover.

Krikorian demonstrates the use of an in-vitro mRNA degradation system in which reactions included the use of ATP, GTP, etc. The degradation of mRNA was then observed and results tabulated. Krikorian further teaches an in-vitro mRNA degradation system comprising a cell extract of lysed HeLa cells (page 113, col.1, "Materials and

Methods") and exogenously added GAPD mRNA as the target mRNA. Cytoplasmic extracts were prepared from infected HeLa S3 cells (page 114, col.2, "Strategy").

Bernstein does not teach the monitoring of deadenylation and degradation of target mRNA.

Brewer demonstrates a system and method for monitoring deadenylation and degradation of target RNA and teaches that poly(A) shortening precedes degradation of mRNA with AU-rich sequences at the 3' end.

Bernstein does not teach a kit comprising comprising a cytoplasmic extract supernatant from a 100,000 x g, 1 hour centrifugation that is depleted of activity of proteins that bind polyadenylate.

Bernstein does not demonstrate the role of other RNA binding proteins in mRNA stability.

Chen describes AU-A and hnRNP A1 proteins as binding to AREs in studies and describes the disruption of functional ARE-protein complex formation as leading to mRNA stabilization.

Zhang teaches the binding of AUF1 (an AU-rich element RNA-binding protein to an AU-rich element in the 3' untranslated region of mRNAs and provides data on the role of this protein in mediating ARE-directed mRNA degradation.

Myer teaches the binding of HuR to mRNA as a protein in AUUUA-mediated mRNA decay and playing a role in regulation of mRNA degradation.

Nakagawa teaches AUG, a gene that codes a protein that binds specifically to AU-rich transcripts.

Levine teaches Hel-N1, an RNA-binding protein with 3' UTR mRNA specificity and requiring a sequence containing AUUUG, AUUUA, and GUUUU.

Nagy teaches glyceraldehyde-3-phosphate dehydrogenase, a protein that selectively binds AU-rich RNA and suggests its role in the regulation of ARE-dependent mRNA turnover.

Nakamaki teaches hnRNP C and AUF1 as AU-rich element binding proteins and determines that AU-binding factors, including hnRNP C and AUF1, may be involved in rapid degradation of mRNA transcripts.

Liu teaches Hu antigens coded by HuD, HuC and Hel-N1 genes are homologues of Elav proteins and bind to AU-rich elements of mRNAs that regulate cell proliferation.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to prepare the mRNA decay system of Bernstein and to further obtain the supernatant by centrifugating at high speeds (100,000 x g for one hour) because doing so would result in successful separation of components as per the teachings of Beaumont. Further, it would have been obvious to one of ordinary skill in the art to utilize ATP, GTP, etc in the method because polyadenylation is an ATP-dependent process as per the teachings of Bernstein and Krikorian teaches the use of the mRNA turnover system in the presence of ATP, GTP, etc. This demonstration in conjunction with the teachings of Bernstein that polyadenylation is an ATP-dependent process would have provided one of ordinary skill in the art with the motivation to perform experiments in the presence of the necessary nucleotide triphosphate component. Krikorian further demonstrates the addition of agents into the mRNA

stability testing system to determine the extent of RNA turnover and utilizes cell extract from HeLa cells. Deadenylation and degradation occur together in mRNA decay as demonstrated through the teachings of Brewer. Brewer teaches the relationship between deadenylation and degradation and demonstrates that deadenylation precedes degradation as well as methods in evaluating deadenylation and degradation of mRNA. This would have provided one of ordinary skill in the art with the motivation to evaluate the deadenylation and degradation of a target RNA sequence utilizing the system and method of Bernstein because deadenylation was demonstrated to be a predecessor of degradation. Also, one of ordinary skill in the art would have been further motivated to utilize the method and system of Bernstein and Beaumont to evaluate the effect of additional ARE RNA binding proteins in the system of evaluating mRNA deadenylation and degradation because the system had been used for evaluating one such protein by Bernstein with the conclusion that ARE RNA binding proteins affected mRNA stability. Further, Chen described a potential mechanism for altering mRNA stability by proteins that bind to the ARE of mRNA and cites two proteins in particular (AU-A and hnRNP A1) that have affinities for the ARE of mRNA and alter mRNA stability. The mRNA instability system and method of Bernstein was used for analyzing the effect of binding proteins and analysis on PABP (poly(A)binding proteins) was performed. Zhang, Myer, Nakagawa, Levine, Nagy, Nakamaki, and Liu each demonstrate the binding patterns of various RNA binding proteins (AU-A, hnRNP A1, AUF1, HuR, AUH, Hel-N1, Glyceraldehyde-3-phosphate dehydrogenase, hnRNP C, HuD, and HuC) and their roles in binding to ARE of mRNA and affecting mRNA stability. It would have been prima

facie obvious to one of ordinary skill in the art to utilize the system and method outlined by Bernstein wherein an RNA binding protein that binds to ARE in mRNA was analyzed as to its effects on mRNA stability and test other known RNA binding proteins that have the same function as the protein analyzed by Bernstein.

Further, one of ordinary skill in the art would have been motivated to package the cell extract and reagents of Bernstein into a kit because assembling the components necessary to perform the methods of Bernstein would have resulted in increased convenience and ease of use of methods requiring those components. Thus, one of ordinary skill in the art would have been motivated to perform the claimed invention with a reasonable expectation of success.

Thus, for reasons stated above, one of ordinary skill in the art would have been motivated to perform the method of Bernstein and Beaumont and to further utilize HeLa cell extract in testing the turnover of mRNA in view of the teachings of Krikorian and to utilize the system for monitoring deadenylation in monitoring deadenylation and degradation as in the teachings of Brewer.

Applicant's arguments filed October 30, 2000 have been fully considered but they are not persuasive. Applicant states that the instant inventors' cytoplasmic extract is a supernatant from at 100,000 xg, 1 hour centrifugation which is depleted of polysomes and therefore differs from the teachings of Bernstein. However, polysomes are removed from the supernatant in the teachings of Bernstein and Beaumont provides motivation for centrifugation at 100,000 x g for one hour as described above. Applicant points out that Bernstein does not achieve "regulated" mRNA degradation. By the term "regulated",

it is construed that the system must bring order, method, or uniformity to degradation of mRNA. However, Bernstein describes the process in which intermediates undergo a stepwise decay pathway in which the poly(A) is first shortened and then removed and then the deadenylated mRNA is then completely destroyed (page 661, right-hand column, lines 37-41). This indicates order to the decay process and thus, the process is "regulated". Applicant further states that Brewer indicates uncertainty as to the relationship of deadenylation and degradation as shown through the statements in the Brewer references that "we do not know what types of nucleases are responsible for either step" and "[p]erhaps the major question raised by our experiments concerns the link between poly(A) removal and mRNA degradation". However, Brewer conducts experiments and concludes that "there is a correlation between poly(A) shortening and degradation" (page 1704, left column, lines 12-13) and that "although there is some controversy ... most published evidence supports ... that poly(A) excision precedes mRNA degradation" (page 1704, right column, lines 3-6). Thus, Bernstein, as well as Brewer, demonstrate regulated deadenylation and degradation to the extent that there is order and method to the process.

Applicant points out the Krikorian does not teach centrifugation at 100,000 x g for one hour. However, Beaumont teaches the effectiveness of centrifugation at the given rate for one hour with successful separation of components within a biological sample. Thus, with the combination of teachings of Bernstein, Beaumont, Krikorian and Brewer, one of ordinary skill in the art would have been motivated to perform the claimed invention as stated above. It was further submitted by the Applicant that the present

invention is not dependent on a particular source of exogenous RNA added to the system, which may be from any source and states that because of this, the present invention differs from the teachings of Krikorian. However, the method as disclosed by Krikorian is not taught to be necessarily limited to any particular source of exogenous RNA. Even if it were assumed to be implied in the teachings of Krikorian that there was some limitation, the invention as claimed in the present invention would still read on the prior art teachings of Krikorian because the method of Krikorian meets the limitations of the claims in question.

In addition, one of ordinary skill in the art would have been motivated to evaluate the effect of additional ARE RNA binding proteins in the system of evaluating mRNA deadenylation and degradation because the system previously used for evaluating an ARE RNA binding protein affecting mRNA stability by Bernstein in view of Beaumont was demonstrated. Other RNA binding proteins and their roles in binding to ARE of mRNA and affecting mRNA stability was taught by any one of Zhang, Myer, Nakagawa, Levine, Nagy, nakamaki, and Liu thus providing the motivation to one of ordinary skill in the art to analyze effects on mRNa stability with other known RNA binding proteins that have the same function as the protein analyzed by Bernstein.

Applicant also states that the prior art differs from the present claimed invention because the present invention includes the deadenylase enzyme and excludes components which non-specifically cleave poly(A) and/or disrupt or inhibit regulated deadenylation and degradation whereas the prior art cell extract includes non-specific exonucleases and other components. However, the claims do not recite limitations on

the presence or absence of "non-specific exonucleoases" or "other components", nor are any excluded "other components" defined in the scope of the claims. The limitation recited in the claim as being depleted of activity of proteins that bind polyadenylate is met by the prior art (Bernstein – see abstract).

In regards to claim 47, Applicant states that the amendment to focus the claim on regulated deadenylation and degradation of a target mRNA molecule renders the claim nonobvious over the prior art. However, as discussed above, the system as disclosed by the prior art brings order and method to degradation of mRNA and as such would constitute "regulated" deadenylation and degradation of a target mRNA.

Finally, Applicant has traversed the rejection of claims 53 and 54 on the grounds that the cytoplasmic extract is described in the present application as being a supernatant of a 100,000 x g, 1 hour centrifugation and is depleted of activity of proteins that bind polyadenylate. However, as discussed above, it would have been obvious to one of ordinary skill in the art at the time the invention was made to centrifuge the sample at 100,000 x g for one hour as doing so would provide effective separation of components in a biological sample as per the teachings of Beaumont.

### *Conclusion*

Claims 1, 2, 4-6, 9-17, 21-47 and 51-55 are rejected. Claims 18-20, 48-50 are allowable.

### *Inquiries*

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Stephen Siu, whose telephone number is (703) 308-7522. The Examiner can normally be reached from 7:00 a.m. to 3:30 p.m. on weekdays. If attempts to reach the Examiner by telephone are unsuccessful, the

Examiner's supervisor, Michael Woodward, can be reached at (703) 308-4028. Papers related to this application may be submitted to Art Unit 1631 by facsimile transmission. The faxing of such papers must conform with the notice published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 CFR 1.6(d)). NOTE: If applicant does submit a paper by FAX, the original copy should be retained by applicant or applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED, so as to avoid the processing of duplicate papers in the Office. The Fax number is (703) 308-0294. Please call the Examiner at (703) 308-7522 before the transmission to expedite delivery of the fax. Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Stephen Siu

JOHN S. BRUSCA, PH.D
PRIMARY EXAMINER